[Frontiers in Bioscience 14, 2307-2334, January 1, 2009]

Integrins and proximal signaling mechanisms in cardiovascular disease

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1. ABSTRACT

Integrins are heterodimeric cell-surface molecules, which act as the principle mediators of molecular dialog between a cell and its extracellular matrix environment. In addition to their structural functions, integrins mediate signaling from the extracellular space into the cell through integrin-associated signaling and adaptor molecules such as FAK (focal adhesion kinase), ILK (integrin-linked kinase), PINCH (particularly interesting new cysteine-histidine rich protein) and Nck2 (non-catalytic (region of) tyrosine kinase adaptor protein-2). Via these molecules, integrin signaling tightly and cooperatively interacts with receptor tyrosine kinases (RTKs) signaling to regulate survival, proliferation and cell shape as well as polarity, adhesion, migration and

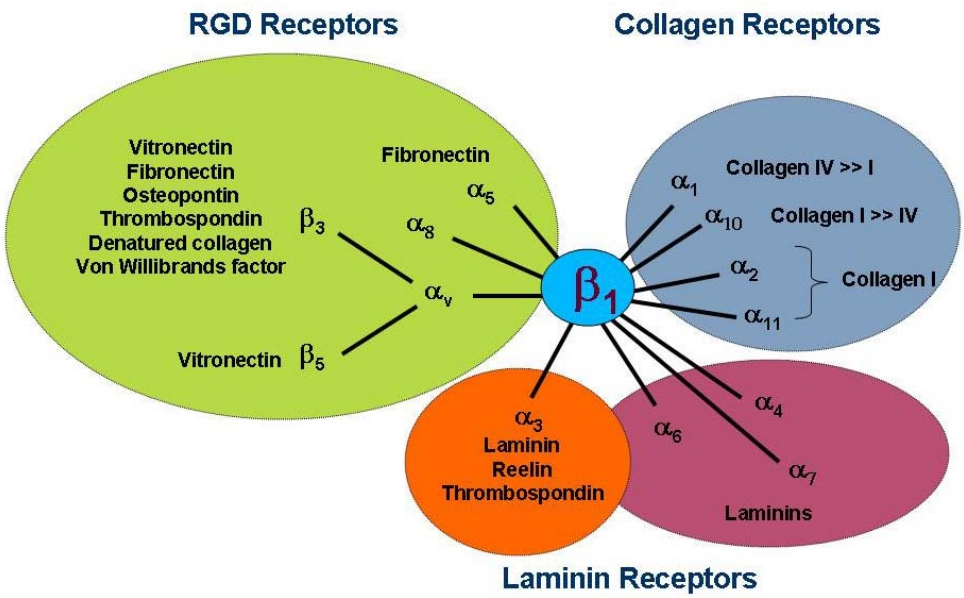

Figure 1. beta $_1$-Integrin Receptors and Ligands. Based on their association in heterodimers, the integrin family can be divided in the beta$_1$, alpha$_V$ and beta$_2$ subgroups. beta$_1$ and alpha $_V$ members are ubiquitously expressed; the beta$_2$ subgroup is selectively expressed in leukocytes. Bars connecting alpha and beta subunits indicate known heterodimers. Ligands for each integrin heterodimer are shown.

differentiation. In the heart and blood vessels, the function and regulation of these molecules can be partially disturbed and thus contribute to cardiovascular diseases such as cardiac hypertrophy and atherosclerosis. In this review, we discuss the primary mechanisms of action and signaling of integrins in the cardiac and vascular system in normal and pathological states, as well as therapeutic strategies for targeting these systems (1).

2. INTRODUCTION

Integrins are a superfamily of heterodimeric cell surface receptors involved in cell–cell and cell–matrix adhesion (2). In addition to providing a direct link between the extracellular matrix (ECM) and cytoskeleton, integrins also regulate the production of second messengers within the cell. As multi-functional molecules, integrins are involved in organogenesis, anchoring of stem cells to niches, regulation of gene expression, cell proliferation, differentiation, migration and death (2-8). An important function of integrins is the ability to convert mechanical forces into biochemical signals (7, 9-18). Because dysregulation of integrins is involved in the pathogenesis of several disease states including atherosclerosis and cardiac hypertrophy (5, 19), extensive efforts have been directed toward understanding how integrins couple to signal transduction systems and integrate with other receptor systems.

2.1. Integrin structure and function

Cell adhesion molecules of the integrin family consist of 18 alpha and 8 beta subunits, which form 24 known alpha-beta-heterodimers depending on cell type and cellular function. Each integrin subunit has a large extracellular, a short transmembrane and small intracellular domain with a total of >1600 amino acids. Integrins are the main receptors for extracellular matrix proteins like collagen, fibronectin and laminin (Figure 1). The alpha subunit generally confers ligand specificity (20, 21) and the beta subunit is important for interacting with the cytoplasmic environment. Cell–matrix interaction via integrins is essential for embryonic development, as well as proliferation, survival, adhesion, differentiation and migration of cells. Ligand binding to the extracellular integrin domain induces conformational changes and integrin clustering for activation of signaling cascades and recruitment of multiprotein complexes to focal adhesions (3, 9). Integrins transmit signals through a variety of intracellular protein kinases and adaptor molecules such as ILK, FAK, talin, paxillin, parvins, p130Cas, Src-family kinases and GTPases of the Rho family. Several integrin subunits like alpha$_1$, alpha$_5$, beta$_1$ and beta$_3$ have been directly implicated in the cardiac pathophysiology, however, the underlying molecular mechanism and downstream signaling cascades are poorly understood (22-25) (Table 1).

2.2. Integrin expression in the cardiovascular system

In the cardiovascular system, integrins are expressed in cardiac myocytes and fibroblasts as well as cells composing the vasculature, blood and neurons. The importance of integrin regulation and function in cardiac cells is given in the following sections.

2.2.1. Cardiac myocytes

Integrin function is required for proper cardiac development and myocyte attachment to extracellular matrix, growth and viability (26). Integrin-dependent

Table 1. Cardiac specific integrin signaling related transgenic animal models and their phenotypic outcome

Promoter/transgene	Cardiac Phenotype	Reference
$Beta_1$ integrin, (cardiac myocyte-specific excision)	Myocardial fibrosis, depressed leftventricular contractility and relaxation, intolerant of hemodynamic load, developed dialated cardiomyopathy by 6 months of age.	(22)
Truncated $alpha_5$ integrin (cardiac-specific gain- of-function)	Conditional expression of a heart-specific truncated $alpha_5$ integrin revealed an 80% reduction in amplitude of the QRS complex, profound systolic dysfunction, decreased connexin-43, loss of gap junctions and abnormal intercalated discs after four days of expression with preserved myocyte contractility and Ca^{2+} transients. This suggests that integrins regulate both mechanical and electrical coupling in the adult heart and that dysregulated integrin activation leads to contractile dysfunction and arrhythmias.	(23)
$Alpha_5$-integrin (wild-type) and $alpha_{5\text{-}1}$ (cardiac specific gain of function)	Cardiac-specific over-expression of the wild-type $alpha_5$-integrin had no detectable adverse effects in the mouse, whereas expression of $alpha_{5\text{-}1}$-integrin [constitutively active] caused electrocardiographic abnormalities, fibrotic changes in the ventricle, and perinatal lethality.	(24)
$Beta_3$ Integrin ($beta_3$(-/-) mice)	$Beta_3$ null mice developed moderate spontaneous cardiac hypertrophy associated with systolic and diastolic dysfunction, which were exacerbated by transverse aortic constriction. The mice also developed mild cardiac inflammation with infiltrating macrophages at baseline, that worsened by left-ventricular pressure-overload. This study suggests that blood-borne cells were partially responsible for the cardiac hypertrophy and inflammation observed in this animal model.	(25)
ILK, (targeted deletion in murine heart)	Targeted ILK ablation in cardiac myocytes resulted in spontaneous cardiomyopathy and heart failure by 6 weeks of age. This these suggests that ILK protects the mammalian heart against cardiomyopathy and heart failure via activation of AKT.	(158)
Transgenic mice expressing cardiac-specific ILKS343D (constitutively active), ILK^{WT} (ILK wild type), ILK^{R211A} (ILK kinase dead)	Transgenic mice expressing constitutively active or wild-type ILK exhibited a compensated ventricular hypertrophic phenotype. In contrast, mice expressing kinase inactive ILK were unable to mount a compensatory hypertrophic response to Ang II. These results suggest that ILK signaling mediates a broad adaptive hypertrophic response.	(284)
FAK (cardiac-specific inactivation)	Transgenic mice expressing inactive FAK have elevated expression of hypertrophic markers, fibrosis with increased expression of collagens I and VI, and develop eccentric hypertrophy upon stimulation with Ang II or pressure overload.	(131)
FAK (cardiac-specific inactivation)	Inactivation of FAK had no effect on basal cardiac performance, myocyte viability, or myofibrillar architecture. However, increases in left ventricular posterior wall thickness, myocyte cross-sectional area, and ANP expression were abolished with left ventricular pressure overload.	(132)
Caveolin-1 knockout mice	Cav-1 null hearts had significantly enlarged right ventricular cavities and thickened left-ventricular walls with decreased systolic function, associated with hypertrophy, fibrosis and hyperactivation of the ERK cascade.	(86)
Caveolin-3 knockout mice	At four months of age, Cav-3 null hearts displayed significant hypertrophy, dilation, and reduced fractional shortening, marked cardiac myocyte hypertrophy, with accompanying cellular infiltrates and progressive interstitial/peri-vascular fibrosis associated with hyperactivation of the ERK cascade.	(87)
Caveolin-1/3 double knockout mice	Cav-1/3 null mice developed severe cardiomyopathy. At 2 months of age, Cav-1/3 null hearts shows a dramatic increase in left ventricular wall thickness and dilation of the left ventricle, with a significant decrease in fractional shortening as compared with Cav-1-KO, Cav-3 KO and wild-type mice.	(88)
Rac1 (cardiac-specific deletion)	The hearts of c-Rac1 null mice showed decreased NADPH oxidase activity and myocardial oxidative stress in response to Ang II stimulation, which was correlated with decreased myocardial hypertrophy. This study suggests a critical role for Rac1 in the hypertrophic response.	(207)

pathways also mediate hypertrophic responses to mechanical stimuli associated with cardiac myocyte strain (10, 11) and are required for stimulation of hypertrophy by phenylephrine (PE) or endothelin-1 (ET-1) (27-29). Cardiac myocytes express integrins $alpha_1$, $alpha_3$, $alpha_5$, $alpha_6$, $alpha_7$, $alpha_9$, $alpha_{10}$, $beta_1$, $beta_3$, and $beta_5$ (7), many of which are regulated by developmental and pathological stimuli (30, 31). Adult myocytes express the laminin binding $alpha_7$ $beta_1$ heterodimer as the major integrin, while the $alpha_5$ $beta_1$ fibronectin receptor and the $alpha_6$ $beta_1$ laminin receptor are expressed in cardiac myocytes during embryonic development (32, 33). The primary beta integrin subunit found in myocytes is $beta_1$. Different splice variants are expressed in the embryonic ($beta_{1A}$) and adult myocytes ($beta_{1D}$) (34), which differ in specific amino acid sequences at the cytoplasmic domain and their interaction with cytoskeletal and signaling molecules (35).

2.2.2. Cardiac fibroblasts

The interaction of cardiac fibroblasts with the surrounding matrix is critical for repair mechanisms, including synthesis of matrix proteins, proliferation, collagen gel contraction and cell motility (36, 37). Cardiac fibroblast activation in the failing heart is associated with increased expression of extracellular matrix proteins (38-41). Cardiac fibroblasts express integrins $alpha_1$, $alpha_2$, $alpha_3$, $alpha_5$, $alpha_8$, $alpha_{10}$, $beta_1$, $beta_3$ and $beta_5$ (37, 42-45). Angiotensin II (Ang II) and other growth factors stimulate cardiac fibroblast contraction and adhesion via $beta_1$ and $alpha_v$ $beta_3$ integrins, which involve inside-to-outside signaling mechanisms (37, 43-45).

3. INTEGRIN BIDIRECTIONAL SIGNALING ACROSS THE PLASMA MEMBRANE

The term "integrin" was coined to reflect the capacity of a receptor family to integrate the extracellular and intracellular environment by bidirectional signaling (Hynes, 1987 #496). Interactions with extracellular matrix (ECM), cytoskeletal and various signal transduction cascades enables integrins to mediate both outside in and inside-out signaling (2, 7, 46). Inside-out signaling occurs when specific intracellular signals impinge on integrin cytoplasmic domains, triggering changes in conformation and ligand-binding affinity in the extracellular domain. For example Ang II induces a significant increase in β_1-integrin-dependent adhesion of cardiac fibroblasts to collagen I (37), by inducing a high-affinity state in the integrin molecule. In turn, binding of extracellular ligands produces intracellular signals (ie, outside-in signals) such as changes in intracellular signaling events and cytoskeletal reorganization that critically influence cell shape, migration, growth, and survival (Hynes, 2002 #1). There is

number of excellent full reviews dedicated to basic mechanism of integrin bidirectional signaling (2, 47, 48). The specificity of integrin signaling is made possible by alpha and beta subunits that form the heterodimeric pair. Analysis of the amino acid sequences of cytoplasmic tails reveals considerable diversity among integrin subunits (49), suggesting differences in signal transduction among these ECM receptors. Because integrins lack enzymatic activity, activation of signal factors requires interaction with cellular proteins that have kinase activity. The cytoplasmic tail of the beta subunit directly binds to several cytoskeletal proteins that associate with signaling molecules (50). Integrins signal through a wide array of intracellular second messenger systems including calcium channels, phosphatidylinositol-4,5-bisphosphate, phospholipase-C (PLC), the Na/H antiporter, tyrosine and serine/threonine kinases, phosphatases, Rho GTPases, mitogen-activated protein (MAP) kinases, and cyclin D1 (13, 51-57).

3.1. Mechanical load and integrin activation

In blood vessels and cardiac cells, shear stress and stretch are important activators of integrins and signal transduction pathways. Mechanical load applied to integrin ligands (ECM) triggers the assembly and growth of focal contacts (58, 59) and activation of FAK and MAP kinases (18, 60). Although integrins works as "receptors" inducing multiple biological functions, the transduction processes are poorly understood. Stretch-induced conformational changes in the ECM may alter integrin structure, resulting in activation of liganded integrin receptors and focal contact-associated secondary messenger pathways in the cell, such as FAK, Src family kinases, Abl and ILK (50, 61). However, other mechanisms may be operational. For example, membrane-bound proteins such as the ADAMs (a disintegrin and a metalloproteinase domain) also support integrin-mediated cell adhesion since these molecules can cleave ECM proteins by their metalloproteinase domains (62). In addition, mechanical stimulation increases growth factor shedding into the ECM (63). Because ADAMs have the ability to shed many cell-adhesion molecules and cell-surface proteins including cytokines and growth factors, signaling pathways activated by these factors could interact with those of integrins. Integrin signaling may also cross-talk with signals generated by stretch-induced secretion of autocrine factors. Uniaxial stretch stimulates autocrine release of Ang II and ET-1, which induce cellular hypertrophy via phosphorylation cascades (64, 65). Interestingly, AT_1 mediates cardiac hypertrophy by upregulation of $beta_1$ integrin expression (66) and acts as a mechanoreceptor in cardiac tissue (67).

3.2. Lipid microdomains

A considerable amount of integrin signaling involves lipid domains located on the cell surface. These microenvironments function as signal organizing centers and platforms by exploiting multiple protein–lipid and protein–protein interactions to link the cytoplasmic tail of transmembrane receptors with other protein scaffolds. These interactions serve to assemble kinases, phosphatases, and other catalytically active molecules in order to generate specific signals that are temporally and spatially controlled (68-70). Integrins signal through at least two types of microdomains, lipid rafts and caveolae.

3.2.1. Lipid rafts

Lipid rafts are specialized plasma membrane structures that have an altered lipid composition and link to the cytoskeleton. These are flat microdomains (71) in the nano-scale range (72, 73) and lack the protein caveolin (69). Lipid rafts that are membrane binding sites for a number of signaling factors, are enriched in lamellipodia and required for cell spreading (74-76). Proteins are targeted to rafts by post-translational modifications, such as glycosylphosphatidylinositol (GPI) anchors or acylated chains, or recruited through the establishment of protein-protein interactions in response to a stimulus (8). In this capacity, lipid rafts coordinate the spatiotemporal organization of signaling components and promote membrane compartmentalization by concentrating integrins, integrin-associated proteins, transmembrane-4 superfamily proteins, GPI-anchored receptors and palmitoylated signaling proteins: H-Ras, Src family kinases and endothelial nitric oxide synthase (eNOS) (77). Because rafts regulate signal transduction and cell behavior, abnormal alterations of these lipid microdomains may contribute to the development of cardiovascular disease.

3.2.2. Caveolae

Caveolae are specialized rafts that contain caveolin and exist as vesicular invaginations of the plasma membrane and plasmalemmal vesicles on the order of 50-100 nm in size (78-80). These structures are highly abundant and critical for signaling in the cardiovascular system. In addition to clathrin-independent membrane traffic (81, 82), caveolae function as organizers of signaling complexes (79, 83), which provides the cell with a mechanism for regulating the "on-off" state of an entire signaling circuit, without further assembly of its components.

Caveolae function varies among tissues due to the various types and amounts of caveolin and the subcellular localization of possible associating partners. Caveolin is a 21 kDa protein first identified as a substrate for the v-Src tyrosine kinase, which, among several other kinases, phosphorylates caveolin on Tyr^{14} (84, 85). Three isoforms of caveolin, Cav-1, Cav-2 and Cav-3, have been described. Cav-1 and Cav-2 are co-expressed and found in most cells, whereas Cav-3 is muscle specific (79). Both Cav-1/Cav-3 and double-knockout mice develop cardiac hypertrophy and associated with hyper-activation of the ERK-MAP kinase cascade (86-88) (Table 1). Cav-1 functions as a "master regulator" of signaling molecules in caveolae and is important for coupling $alpha_1$ integrin to activation of Shc and ERK (15, 89-91) in vascular smooth muscle cells and cardiac myocytes. The caveolins form the coat material and decorate caveolar necks (92). Caveolin binding is mediated by a "membrane-proximal region" of caveolin, which has been termed the caveolin scaffolding domain (93). Through this domain, caveolins bind to many classes of signaling molecules, including integrins, heterotrimeric G-protein alpha subunits, Ras, Src family kinases, eNOS, RTKs and protein kinase C (PKC) isoforms

(79). Caveolins provide a domain, which specifically interacts with a variety of proteins. This domain has a variable amino acid sequence shown to be ΦXΦXXXXΦ or ΦXXXXΦXXΦ where Φ is W, F, or Y (94). In addition to concentrating signal transducers to regions of the plasma membrane, caveolin binding may regulate the activation state of caveolae-associated signaling molecules. Because signaling proteins associated with caveolin are in an inactive state, this may prevent inappropriate activation by gathering components of signal transduction in a spatially defined compartment. However, if activation of a given signaling pathway occurs and proper signaling ligands are available, then sequestered molecules can dissociate from caveolin and leave the caveolae (83).

3.3. Actin-integrin adhesion complexes

In the past 30 years, focal adhesions (focal contacts or matrix adhesions) have been used to study cell-matrix interactions. Specific transmembrane adhesion receptors, such as integrins and several other cytoskeletal proteins have been found localized within these regions. Examination of these adhesion sites have identified a variety of actin-integrin adhesion complexes (AIACs), which vary according to composition and function. In this capacity, AIACs mediate 2-way crosstalk between the extracellular matrix and the cytoskeleton and serve as important focal points for signal transduction processes. Integrins nucleate three distinct types of focal contacts, which represent different stages in the interaction of cells within the matrix. These major variants are focal complexes, focal adhesions and fibrillar adhesions (58, 95). AIAC diversity is regulated locally and globally at multiple levels by actomyosin contractility, as well as by a variety of signaling molecules such as Src, Rho GTPases, MAP kinases and FAK. Thus, AIACs are dynamic signaling components, which reflect the current status of structure and function within a tissue.

3.3.1. Focal Complexes

Focal complexes are “dot-like” structures located at the edge of lamellipodia and contain paxillin, vinculin and tyrosine-phosphorylated proteins. Focal complexes are early adhesions, which transform into focal adhesions, following the action of RhoA or as a result of external mechanical perturbation (14, 96). The precise role of focal complexes in mediating functional responses in the cardiovascular system remains to be more fully explored. There is evidence to suggest that focal complexes play an important role in the development of cardiac hypertrophy by coupling fibronectin and vitronectin binding integrins ($alpha_v$-$beta_3$ and $alpha_5$-$beta_1$) to activation of MAP kinases (12). Large focal adhesions and small peripheral focal complexes are inversely interconnected (97). This is consistent with the deterioration of contractile function and cytoskeletal reorganization, which occurs in the early stages of cardiac hypertrophy (98). The interaction of fibronectin and Arg-Gly-Asp (RGD)-dependent integrins to form focal complexes in the hypertrophic response, suggests that ECM proteins are not merely passive adhesive molecules, but active participants in processes leading to myocyte hypertrophy.

3.3.2. Focal Adhesions

Focal adhesions are large, flat, elongated structures associated with the ends of actin filament bundles (stress fibers) which are typically located at the cell periphery and contain paxillin, vinculin, alpha-actinin, talin, FAK and tyrosine-phosphorylated proteins. Focal adhesions report to cells regarding physical properties of the surrounding environment and participate in detailed transmembrane cross-talk between the ECM and intracellular signal transduction systems. The focal adhesions also function as mechanosensors (95), which increase in size upon application of force (either cell-generated or external) and shrink upon relaxation, there by physical dimensions proportional to the applied tension. The complexity of focal adhesions is being increasingly revealed. To date, over 50 distinct molecules have been shown to reside within these structures (51, 99).

3.3.3. Fibrillary adhesions

Fibrillar adhesions are located centrally and contain fibronectin and tensin. $beta_1$ integrins can translocate from focal complexes to focal adhesions and ultimately fibrillar adhesions. The fibrillar adhesions arise from focal adhesions following actomyosin-dependent centripetal displacement of ECM-associated fibronectin receptors (100, 101). Fibronectin is a multidomain glycoprotein that plays a role in the wound-healing process by providing a suitable matrix for cells to migrate on and by acting as a chemoattractant that induces cell migration toward the site of myocardial injury (102). It also stimulates fibroblasts in the healing wound to become myofibroblasts, which are important for wound contraction and further fibronectin matrix assembly (103). Mechanical wounding of cultured interstitial valvular cells is associated with secretion of fibronectin at the wound edge and formation of prominent fibrillar adhesions composed of tensin and $alpha_5$-$beta_1$ integrin (104). This suggests that fibrillar adhesions may have a predominant role in repair of mechanically injured cardiac valves.

3.4. Focal adhesion kinase

FAK is a 125 kDa non-receptor protein kinase, which directly binds to the cytoplasmic tail of beta-integrin and plays a major role in integrin-mediated signaling (105). It was discovered 15 years ago as a highly tryrosine-phosphorylated protein that localized to integrin-enriched cell adhesion sites (106, 107). The biological importance of FAK-mediated signal transduction is underscored by the fact that it plays a fundamental role in embryonic development (108, 109), control of cell migration (110-112) and cell cycle progression (113).

3.4.1. Structure and function

The amino acid sequence of FAK is highly conserved among species and the protein structure of FAK protein is identical among humans, mouse and chickens (106, 107, 114-116). FAK consists of three major functional domains (117), which include an N-terminal domain (FERM), a catalytic domain for tyrosine kinase activity, and a FAT (focal-adhesion targeting) domain (Figure 2). The N-terminal domain interacts with integrin and growth factor receptors, whereas the FAT domain

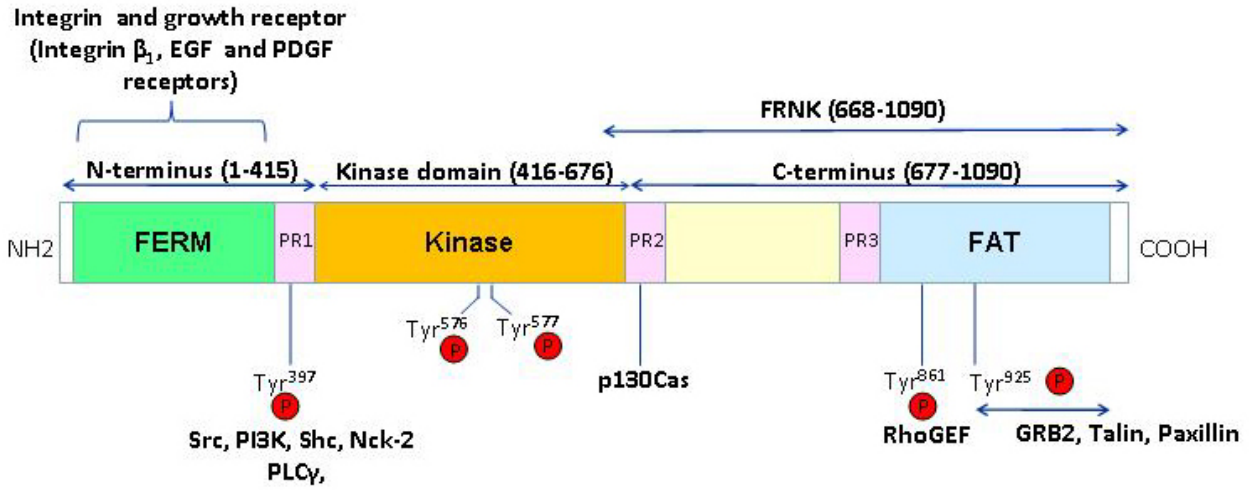

Figure 2. Focal adhesion kinase (FAK) Structural Features and Binding Partners. FAK has the N-terminal, kinase and the C-terminal domain. The centrally located kinase domain is flanked by large N- and C-terminal domains. Important tyrosine phosphorylation sites of FAK are shown. The N-terminal domain has the Tyr^{397} autophosphorylation site. Phosphorylated Tyr^{397} also interacts with Src, PI3K, Shc, Nck-2 and PLCgamma. The kinase domain has the Tyr576/577, critical for FAK kinase activity. The C-terminal domain has Tyr^{861} and Tyr^{925} phosphorylation sites. Integrin associated proteins such as paxillin and talin binds at the FAT (focal adhesion targeting) region in the C-terminal domain. FAK contains three proline-rich regions (PR1-3), which binds to Src-homology-3 (SH3) domain containing proteins such as p130Cas. FAK related non-kinase (FRNK) is a negative acting splice variant of FAK, consisting of amino acids 668-1090, its lacks both the N-terminal and the kinase domain. FAT: focal adhesion targeting; FERM: protein 4.1, ezrin, radixin and moesin homology; Src: Rous sarcoma oncogene cellular homolog; PI3K: phosphatidylinositol 3-kinase; Shc: SH2-containing collagen-related proteins; Nck-2: Nck adaptor protein; Cas: Crk associated substrate; PLCgamma: phospholipase Cgamma; EGF: epidermal growth factor receptor; PDGF: platelet-derived growth factor receptor; GEF: guanine nucleotide-exchange factor; GRB2: Growth factor receptor-bound protein.

localizes FAK to focal adhesions and contains binding sites for a number of signaling molecules including phosphoinositide 3-kinase (PI3K), p130Crk associated substrate (CAS), talin and paxillin. The recruitment and activation of these molecules are important for subsequent activation of other downstream signaling pathways. FAK also contains caspase cleavage sites for the generation of carboxyl-terminal fragments that inhibit FAK phosphorylation and thus act like FAK related non-kinase (FRNK), the naturally occurring variant of FAK (118). Because the FAT domain is preserved in FRNK, it causes a dominant-negative effect on FAK, as well as other FAK-like kinases. Importantly, FRNK is expressed autonomously due to alternative splicing, and could therefore play a potential role in integrin-mediated signaling by negative regulation of FAK.

3.4.2. Expression and regulation

FAK is highly expressed in cardiac myocytes and undergoes phosphorylation at Tyr^{397}, Tyr^{861} and Tyr^{925} in response to mechanical loading (105, 119, 120). In neonatal rat ventricular myocytes, FAK is rapidly activated by cyclic stretch and translocated from the perinuclear area to costameres (119). FAK is also activated by G-protein coupled receptors, such as Ang II (121), ET-1 (122) and PE (123). Thus, mechanical stretches, together with autocrine release of factors activate FAK in cardiac myocytes. Stretch experiments, performed using cardiomyocytes isolated from AT_{1A} receptor knockout mice (124) and with AT_1 receptor blocker (119), indicate that mechanical stretch alone is sufficient to activate FAK signaling. The molecular mechanisms by which FAK is activated by mechanical signals require further exploration. FAK activation could result from integrin activation and/or confirmation changes due to stretching of the FAK molecule, such as in the case of p130Cas (125).

3.4.3. Role of FAK in vascular function

Inhibition of FAK signaling in endothelial cells in culture using dominant-negative, antisense or knock out strategies impairs the ability of the cells to form tubules in matrigel (126). In the mouse, overexpression of FAK in vascular endothelial cells results in increased angiogenesis in both a hind limb ischemia model and wound-induced angiogenesis model (127). Endothelial cells from *fak*$^{-/-}$ mice exhibit a 2-fold increase in apoptosis *in vivo*, when grown in culture suggesting that promotion of cell survival is a key FAK function for angiogenesis during development (128). Interestingly, the mutant cells show reduced proliferation *in vitro* (129), which is not detected in *ex vivo* cultures or *in vivo* (128). This discrepancy of results could reflect cell culture conditions and/or the type of models (e.g. injury model) studied. Additional studies will be required to resolve the role of FAK in vascular proliferation and wound healing.

3.4.4. Role of FAK in cardiac function

Analysis of FAK total knockout embryos, as well as studies using various *in-vitro* systems, suggests a potential role of FAK in heart development and function.

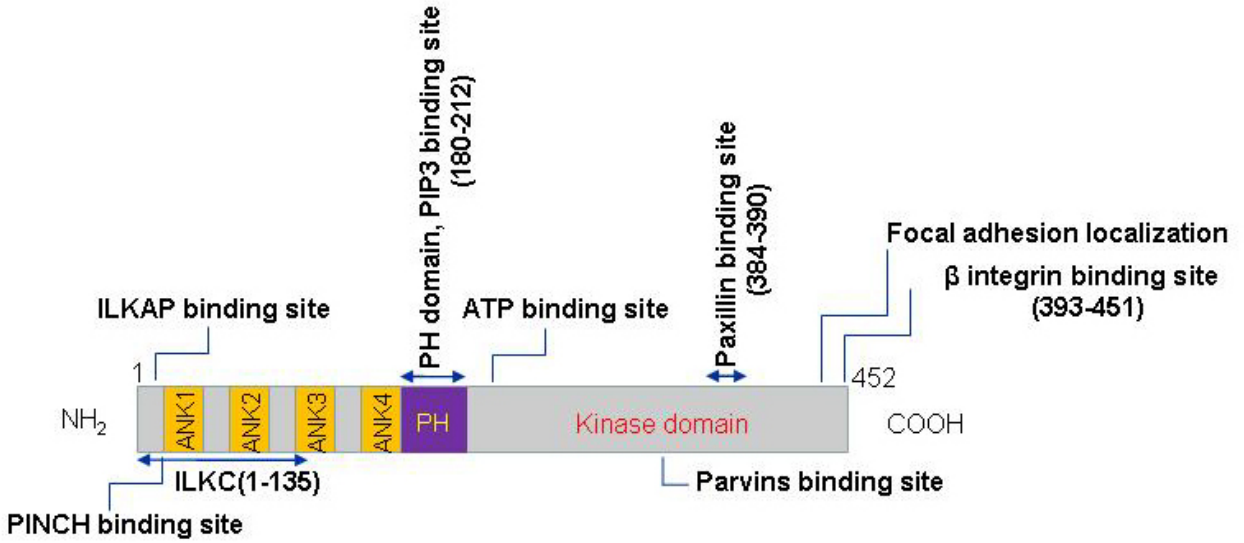

Figure 3. Primary Structure of ILK. Three distinct functional domains are shown. The N-terminal ankyrin repeat and the C-terminal kinase domain flank a plecktrin homology (PH) like domain with conserved motifs for the binding of phosphoinositide phospholipids. All three domains are highly conserved between Drosophila, mice and humans. Figure also depicts the interaction sites for different signaling molecules obtained from number of point mutation and deletion modifications studies. By interacting with these molecules ILK forms a protein complex which functions to link integrins to receptor tyrosine kinases to the actin cytoskeleton and down-stream signaling molecules, thus affecting gene expression. ILK-associated phosphatase (ILKAP) binds to the ankyrin repeats of ILK, is a serine/threonine phosphatase and has been shown to negatively regulate ILK kinase activity. Signaling proteins, AKT/PKB, PDK-1, and GSK-3 can also interact with ILK with in kinase domain and activated ILK can directly phosphorylate AKT at Ser^{473} and GSK-3 at Ser^{9}.

FAK gene inactivation in mice results in a lethal embryonic phenotype with major defects in the axial mesoderm and cardiovascular system (108, 130). Neither a normal heart nor fully developed blood vessels were present in the FAK null embryos. Disruption of endogenous FAK/Src signaling inhibited stretch-induced atrial natriuretic factor (ANF) gene activation, suggesting that FAK plays an important role in load-induced cardiac myocyte hypertrophy. However, in separate mouse study, inactivation of FAK in ventricular cardiac myocytes was found to promote eccentric cardiac hypertrophy and fibrosis in response to Ang II stimulation (131), suggesting that FAK functions to prevent hypertrophy (Table 1). In this study, conditional FAK-KO mice developed spontaneous left-ventricular chamber dilation by 9 months of age. Recently two contradictory *in vivo* studies with myocyte-restricted FAK-inactivated animal model have been published (131, 132). One study advocates that inactivation of FAK promotes eccentric cardiac hypertrophy (131), whereas the other suggests that it attenuates pressure overload-induced hypertrophy (132) (Table 1). Thus, it remains unclear as to whether FAK promotes or prevents cardiac hypertrophy. The precise role of FAK in controlling cardiac hypertrophy will be an important issue to resolve due to its potential clinical relevance.

3.5. Integrin-linked kinase

ILK was initially described as a ubiquitously expressed serine/threonine kinase that binds directly to the cytoplasmic domain of $beta_1$ integrin (133). It is now known that ILK directly interacts with the cytoplasmic domains of both $beta_1$ and $beta_3$ integrins (133, 134) and plays an important role in regulating numerous cellular processes and extracellular matrix accumulation (135, 136). It is highly expressed in cardiac muscle, where it plays an important role in cell migration and the development of cardiac disease related to integrin function (137-140).

3.5.1. Structure and function

The gene encoding ILK has been mapped to human chromosome 11p15.4/15.5 and encompasses 13 exons and 12 introns (135, 136). ILK is a 50 kDa protein (452 amino acids) that exhibits three structurally and functionally distinct domains (Figure 3). The N-terminus of ILK consists of four ankyrin repeats, which are essential for binding to LIM (Lin11, Isl-1, Mec-3) adaptor proteins PINCH-1 and PINCH-2, ILKAP (ILK-associated protein serine/threonine phosphatase of the PP2C family) and localization of ILK to focal adhesions (141, 142). The PINCH-1 and PINCH-2 isoforms each consist of five LIM domains and tandem nuclear localization signals (143). Although all four ILK ankyrin repeats are required for PINCH binding, only the N-terminal LIM domain of PINCH-1 or PINCH-2 specifically interacts with ILK in a mutually exclusive manner (61, 141, 144). The central ILK pleckstrin-homology domain (136, 145) is activated by PIP3 (phosphatidylinositol 3,4,5-triphosphate), whereas the carboxy-terminal kinase catalytic domain of ILK mediates structural interactions with beta integrins, with the actin binding proteins alpha-, beta- and gamma-parvin, thereby providing a connection to the actin cytoskeleton and with the paxillin LD1 motif. ILK forms stable ternary

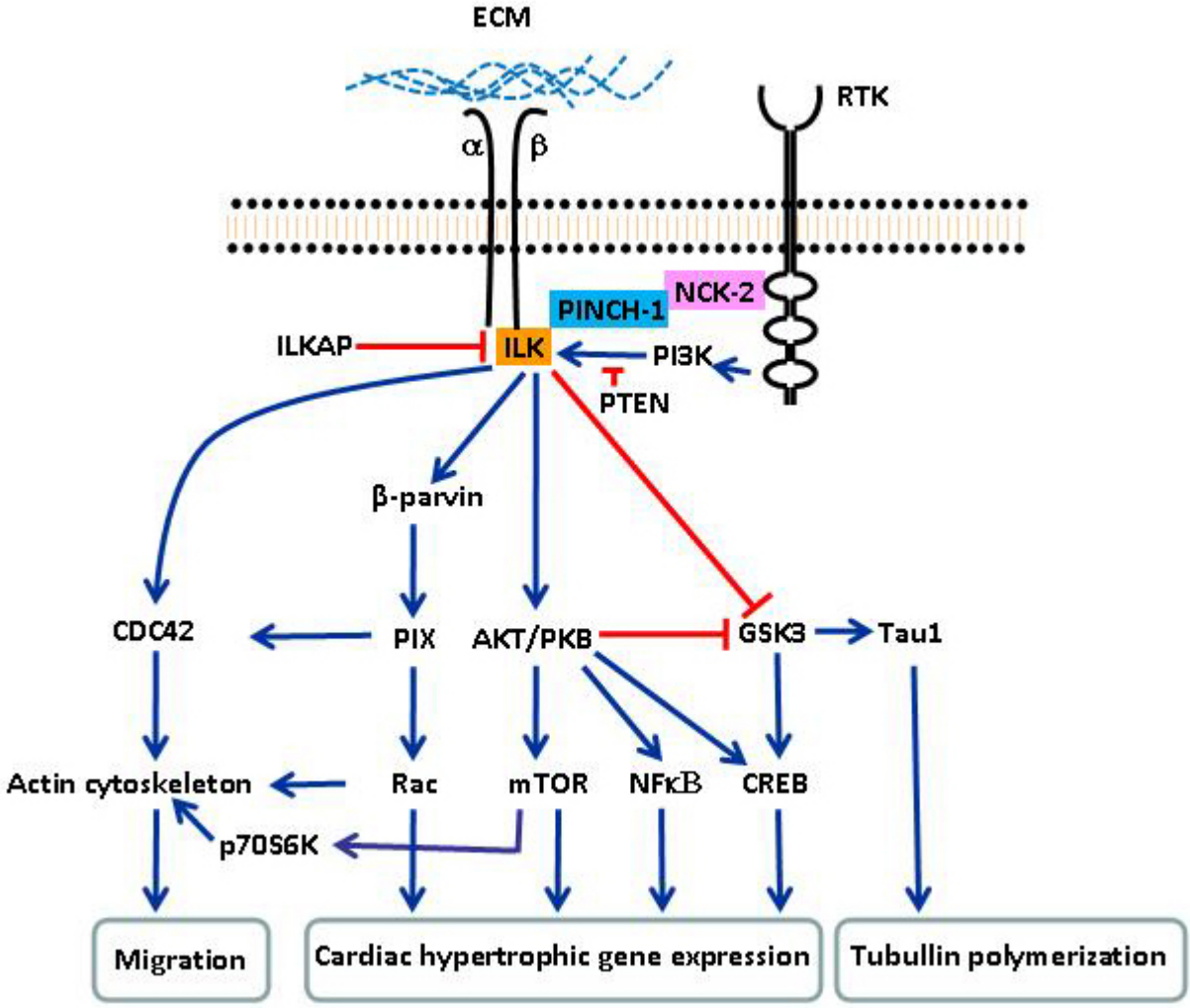

Figure 4. Integrin mediated ILK signaling: ILK forms a complex which functions to link integrins and receptor tryrosine kinases (RTKs) to the actin cytoskeleton and downstream signaling molecules. Integrin and growth factor receptors can activate AKT and GSK3 in an ILK dependent manner. The activity of ILK is upregulated by PI3K and down regulated by the ILK associated phosphatase (ILKAP). PTEN is a negative regulator of PI3K, thus down-regulating the activities of ILK and PKB/AKT. By stimulating the phosphorylation of AKT at serine 473, ILK stimulates several signaling pathways like mTOR, NFkappa-B and CREB, leading to the cardiac hypertrophic gene expression. ILK also stimulates phosphorylation of GSK3 at serine 9, leading to its inhibition that result in the activation of transcrition factor CREB, which is an important regulator of cardiac pathophysiology. PINCH-1; PTEN: Posphatase and tensin homologue deleted on chromosome 10; AKT: AKT8 virus oncogene cellular homolog; GSK: Glycogen synthase kinase; mTOR: Mammalian target of rapamycin; NF kappa-B: Nuclear factor kappa-B; CREB: cAMP response element-binding protein

complexes with intracellular proteins to form a PINCH-ILK-Parvin complex (146, 147), which stabilizes focal adhesions (146-149).

ILK has a low basal activity, which is markedly increased by growth factors and integrin clustering (145). Phosphatidylinositol 3 kinase (PI3K), PIP_3 lipid phosphatase (PTEN), and integrin-linked kinase-associated phosphatase 2C (ILKAP) are upstream regulators, whereby PI3K and PTEN regulate ILK activity by affecting PIP_3 binding to the pleckstrin-homology domain of ILK (142, 150-152). ILK directly couples to protein kinase B (Akt) and glycogen synthase kinase–3-beta (GSK-3-beta) (150, 153, 154). Activated Akt and GSK-3-beta further phosphorylate downstream signaling cascades mTOR, NF-kappaB and CREB, which have been implicated in cardiac cell growth (Figure 4). In addition, ILK can directly and indirectly phosphorylate myosin light chain and contribute to Ca^{2+} sensitization of VSM cell contraction. The mechanism by which ILK couples to these effectors is complex. Recent studies suggest that ILK is more important as an adaptor than a kinase, by recruiting kinases into a multi-protein complex, which in turn phosphorylates Akt and GSK-3-beta (155-157). However, the importance of catalytic and noncatalytic functions of ILK may be cell-dependent and require further investigation.

In the few studies performed, results indicate that ILK plays an integral role in cardiovascular signaling mechanisms associated with integrin signaling. Targeted ablation of ILK from murine heart in cardiac myocytes results in dilated cardiomyopathy and spontaneous heart failure (158) (Table 1). ILK is most abundant in the myocardium (133), suggesting that plays an important role in transducing integrin-dependent mechanical signaling in cardiac cells. In the heart, ILK appears to primarily activate signaling pathways associated with survival, development and adaptive cardiac hypertrophy (physiologic hypertrophy), rather than maladaptive hypertrophy (pathological hypertrophy) (159). In a pressure-overload mouse model of cardiac hypertrophy, there is a significant increase in ILK mRNA (160). Deletion of ILK in zebra fish using antisense oligonucleotides results in marked patterning abnormalities of the vasculature and is lethal (138). Thymosin $beta_4$ protein regulates cardiac cell migration and survival through activation of ILK. In a coronary artery ligated mouse model, thymosin $beta_4$ treatment resulted in upregulation of ILK and Akt activity in the heart, enhanced early myocyte survival and improved cardiac function (161). For cardiac development, ILK can bind to Mig-2 (a Rac GTPase), and regulate nuclear cardiac transcription factor CSX/Nkx-2.5, to affect cardiac development (162). ILK also plays an important role for cell survival in neonatal rat ventricular myocytes and vascular endothelial cells. Overexpression of a dominant-negative mutant lacking the PINCH-binding motif (ILK-C), or deletion of the ILK gene in cardiac myocyte induces marked apoptosis. Manipulated cardiac myocytes lose the protective effects of fibronectin and PE against apoptosis, induced by hydrogen peroxide or serum deprivation. This suggests that ILK and PINCH-ILK-parvin complexes regulate cardiac myocyte cell apoptosis (163).

3.6. Integrin-mediated Akt activation

In the past decade, several studies have demonstrated that Akt is involved in a variety of cellular functions, including regulation of cell metabolism, motility, survival, apoptosis, hypertrophy and gene transcription. Akt performs these tasks by phosphorylating more than 20 different proteins located in the cytoplasm, mitochondria, and the nucleus. Akt is a homologue of the viral oncogene *v-akt* (164), which is related to PKA and PKC (165). The three known Akt isoforms (Akt1/PKBalpha, Akt2/PKBbeta and Akt3/PKB gamma) are derived from distinct genes. Akt1 and Akt2 are the most abundant Akt isoforms in the heart and the vasculature. Akt1 is required for physiological hypertrophy in response to exercise training and IGF1 stimulation (166). Transgenic overexpression of Akt isoforms in the heart results in a greater degree of cardiac hypertrophy with a broad spectrum of functional consequences from increased contractility to decreased ejection fraction and heart failure, which may depend in part on the degree of Akt overexpression (167-169). The hypertrophic response in heart activated by Akt is similar to that induced by exercise (i.e. physiologic hypertrophy) (170-172). In the vessel wall, the loss of Akt1 increases inflammatory mediators and reduces eNOS phosphorylation, suggesting that Akt1 exerts vascular protection against atherogenesis (173). Also, shear stress promotes differentiation of endothelial progenitor cells via activation of Akt (174).

The mechanisms by which Akt is activated remain to be determined. Recent studies have suggested that the activity of Akt is controlled differently in a stimulus and cell type-dependent manner (175-177). The pleckstrin-homology domain in the N-terminal region of Akt interacts with 3′-phosphoinositides, contributing to recruitment of Akt to the plasma membrane. Recruitment to the membrane results in a conformational change that exposes two crucial amino acids that are phosphorylated and necessary for activation. One is Thr^{308} (in Akt1), which is located in the kinase domain and phosphorylated by constitutively active phosphoinositide-dependent kinase 1 (PDK1), resulting in stabilization of the activation loop. The other is Ser^{473} (Akt1), which is located in the hydrophobic C-terminal domain, which is phosphorylated by PDK2 and necessary for full activation (178, 179). Several potential PDK2s have been identified, including ILK (155), the mTOR rictor complex (but not the rapamycin-sensitive mTOR raptor complex) (180) and PKCbetaII (181). It is not clear how these potential PDK2 molecules may interact to regulate Akt. In addition, the mechanisms by which $beta_1$ integrin activates Akt at Thr^{308} and Ser^{473} in heart and the vasculature remain to be explored. The activation process may involve FAK-dependent (182) and independent (183) mechanisms, depending upon the cellular context.

3.7. Rho Family of GTPases

The low-molecular-weight (20-30 kDa) or small GTPase superfamily consists of more than 100 members, which are broadly grouped based on structural similarities into five subfamilies including (1) Arf/Sar1, (2) Rab, (3) Ran, (4) Ras and (5) Rho/Rac/Cdc42. Ras is the first small GTPase recognized to have an important role in cardiac hypertrophy and has been extensively studied in that context. In the past decade, the Rho family of GTPases, which link integrins and other cell surface proteins to the actin cytoskeleton and orchestrate fundamental cellular processes (184-186), have become recognized as important regulators in cardiovascular system. Each member of the Rho GTPase family serves specific functions in terms of cell shape, motility, secretion and proliferation, although overlapping functions between the members have been observed in over-expressed systems. These signaling functions have been demonstrated be mediated through through actin-dependent and actin-independent mechanisms.

3.7.1. Actin-dependent signaling of Rho GTPases

In response to activation by extracellular ligands, RhoA leads to myosin light-chain (MLC) phosphorylation and formation of focal-adhesion complexes (187). In contrast, Rac activation leads to the formation of lamellipodia and membrane ruffles, whereas Cdc42 activation induces actin-rich surface protrusions called filopodia. These distinct but complementary functions of Rho family members also extend to their effects on cell signaling. Once activated and translocated to specific subcellular locations, Rho proteins interact with

downstream effector molecules to engage specific signaling cascades (188, 189). To date, more than 70 potential effectors have been identified for members of the Rho/Rac family (187). The effects of RhoA and Rac1 on the actin cytoskeleton and cell morphology are mediated through stimulation of downstream effector kinases by the activated (GTP-liganded) Rho protein. For RhoA, the best known effectors are Rho kinase (ROCK) and mammalian diaphanous (mDia). Two isoforms of ROCK (ROCK1 and ROCK2), have been identified (190). Genes expressing ROCK1 and ROCK2 are located on human chromosomes 18 (18q11.1) and 2 (2p24), respectively. Although both isoforms are ubiquitously expressed, ROCK2 is highly expressed in brain and heart, whereas ROCK1 is preferentially expressed in lung, liver, spleen kidney and testis. ROCK phosphorylates the myosin binding subunit of MLC phosphatase, resulting in increased myosin phosphorylation and contraction (191, 192).

3.7.2. Actin-independent signaling by Rho GTPases

RhoA, Rac1, and Cdc42 also affect gene transcription through signal transduction pathways not involving the actin cytoskeleton. All three GTPases are capable of activating the JNK and p38 MAP kinase pathways; however, this depends on the particular cell type (193-195). At least four MAP kinase kinase kinases (MAPKKKs) are direct targets of Rho GTPases: mixed-lineage kinase (MLK) 2, MLK3, and MKK4 interact with Rac1/Cdc42, whereas MAPKK1 interacts with RhoA and with Rac1/Cdc42, although through different sites (196-199). Rac1 and RhoA also regulate other non-actin effectors, such as ion channels, NF- kappaB, other transcription factors and reactive oxygen species generation (186, 200, 201).

3.7.3. RhoA and Rac1 in cardiovascular signaling

Of the 20 known Rho family gene products, RhoA, and Rac1 have been most extensively studied in the context of cardiovascular signaling. In the vasculature, Rho signaling pathways are intimately involved in the regulation of endothelial barrier function, inflammation and transendothelial leukocyte migration, platelet activation, thrombosis and oxidative stress, as well as smooth muscle contraction, migration, proliferation and differentiation, and are thus implicated in many of the changes associated with atherogenesis. Rho-associated protein kinase increases the sensitivity of vascular smooth muscle to calcium in hypertension (202) and coronary spasm (203). In endothelial cells, fibronectin-induced activation of NF-kappaB via $alpha_5$ $beta_1$ involves activation of RhoA and Rac1 and occurs in the absence of concurrent signals from growth factor receptors (204), indicating a direct connection between integrins and gene regulatory signals in the vasculature. RhoA and Rac1 are also involved in pressure overload induced cardiac hypertrophy (205-207) (Table 1). Recent studies have shown that ROCK1 deficient mice preserved compensatory hypertrophic response, but showed reduced perivascular fibrosis and interstitial fibrosis in response to pressure overload (208, 209). Increased ROCK has been observed in a mouse model of myocardial infarction, as indicated by an increase in ezrin/radixin/moesin (proteins which are known downstream targets of Rho-kinase) phosphorylation, fibrosis, hypertrophy and inflammation in the left ventricle following coronary artery ligation (210). Expression of constitutively active Rac1 produces hypertrophic remodeling of cultured cardiac myocytes and dilated cardiomyopathy *in vivo* (211). Rac1 is also required for phenylephrine-induced hypertrophic phenotype in cultured cardiac myocytes (212). Although integrin coupling to Cdc42 has been examined in non-cardiovascular cell types (213), its role remains to be determined in the heart and vasculature.

3.8. Mitogen-activated protein kinases (MAP kinases)

MAP kinase pathways provide important links between integrins and nucleolus via phosphorylation and regulation of multiple transcription factors. On the basis of sequence homology, MAP kinases have been divided into three major subfamilies: extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38. These kinases are ~60–70% identical to each other and differ in the sequence and size of their activation loop, as well as in their activation in response to different stimuli. Each MAP kinase subfamily consists of several isoforms and members, which often have distinct functions. Several ERKs (1-6) are expressed in the heart, of which ERK1 is most abundant (214). JNK and p38 were initially identified as stress-activated protein kinases (SAPKs) (16). Subsequently, these have been shown to belong to different signaling pathways, based on differences in phosphorylation motifs, upstream activators and downstream targets (215). JNK is named after the immediate-early gene c-jun, the first substrate identified (216). The JNKs are encoded by three genes, *jnk1*, *jnk2* and *jnk3*, which are differentially spliced to yield four JNK1 isoforms, four JNK2 isoforms and two JNK3 isoforms (217). Alternative splicing of sequence near the 3′-end of the coding region results in p46 and p54 forms of the three distinct *jnk* gene products. Alternative exon 6 useage, which encodes residues within the protein kinase subdomain IX and X of JNK1 and JNK2, produces the corresponding JNKalpha or JNKbeta isoforms. Only JNK1 and JNK2 isoforms are expressed in the myocardium (215). In the p38 family, four genes give rise to the isoforms p38alpha, p38beta, p38 delta and p38 gamma (218), of which p38 alpha, is the major isoform expressed in the adult myocardium (219).

An extensive number of studies have documented ERK, JNK and p38 activation in the pressure overloaded myocardium and cardiac myocytes exposed to mechanical stress and various types of humoral stimuli (206, 220-224). This initially led to the postulate that all three branches of the MAP kinase pathway are involved in mediating myocyte hypertrophy. However, more recent studies suggest that both ERK (206) and JNK (225) activate anti-hypertrophic signaling pathways in the heart**,** therefore opposing p38 effects on cardiac growth. Interestingly, over-expression of MAP kinase phosphatase-1, which inhibits all the three major branches mentioned above, blocks agonist-induced pressure-overload induced cardiac hypertrophy (226). This indicates significantly different roles for MAP kinase pathways in the cardiac hypertrophic

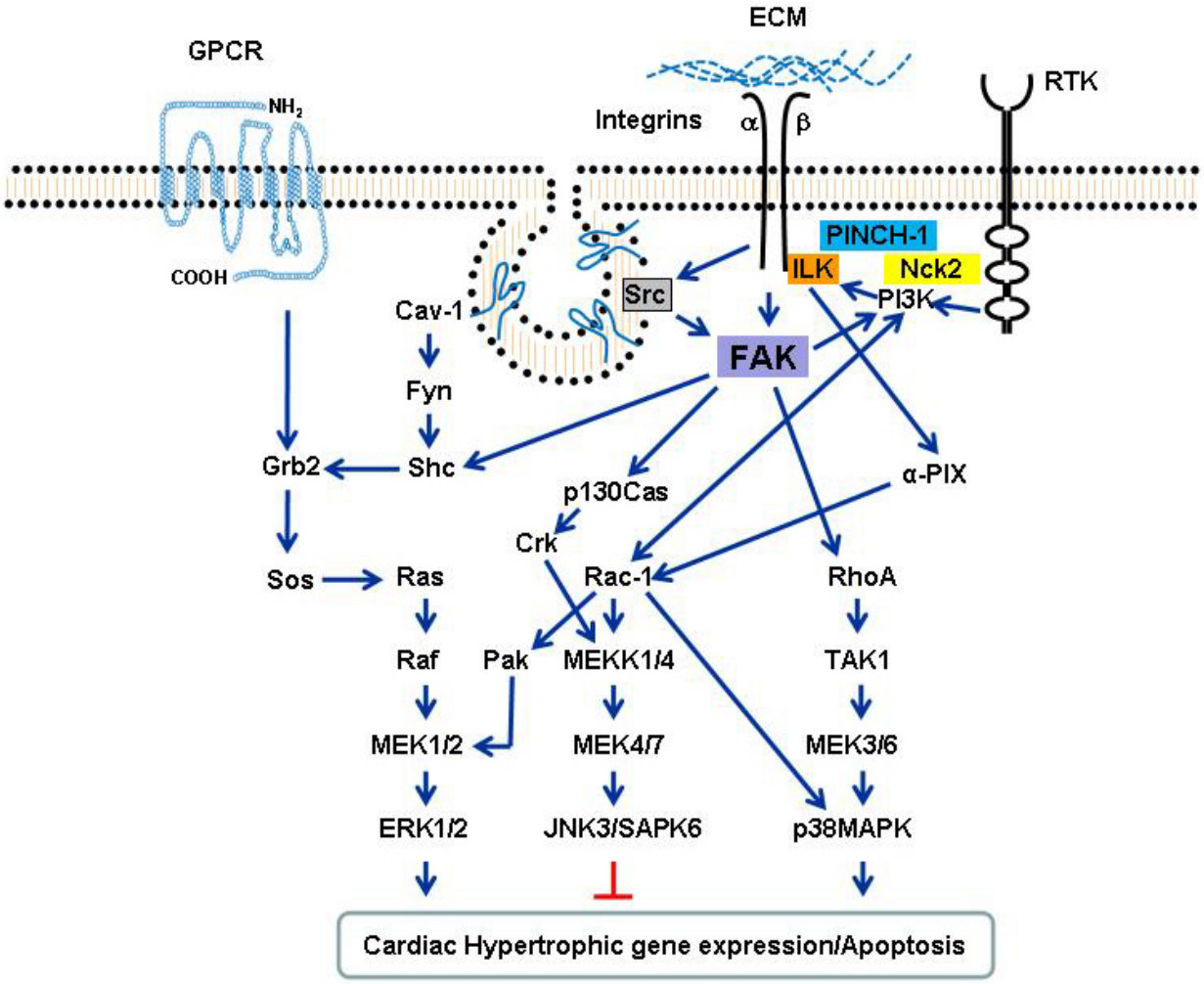

Figure 5. Integrin Mediated Rho GTPase Signaling. Various extracellular stimuli such as harmons, growth factors, neuromediators, interaction with extracellular matrix (ECM) to mechanical stretch activate guanine exchange factors (GEFs) leading to activation of Rho. GTP bound Rho subsequently activates ROCK to phosphorylate several substrates leading to various cellular responses that directly and/or indirectly cause cardiovascular diseases. Thus ROCK inhibitors seem to be useful for treating disorders caused by vascular smooth muscle cell hypercontraction, arteriosclerotic diseases and other diseases. ROCK; Rho kinase, PI3-K; phosphoinositide 3 kinase, eNOS; endothelial nitric oxide synthesis, NO; nitric oxide synthesis, PAI; Plasmogen activator inhibitors, GEF; Guanine nucleotide exchange factor, GAP; GTPase activating protein, ECM; Extracellular Matrix, GPCRs; G-protein coupled receptors, PPI; Protein prenyltransferase inhibitors).

signaling, which remain to be clarified by future studies.

3.8.1. Integrin-induced ERK activation

Integrins activates ERK1/2 via FAK dependent (227) and independent mechanisms (120) (Figure 5). FAK activation and its role in mediating ERK activation are poorly understood. One mechanism involves auto-phosphorylation of FAK Tyr^{397}, creating a binding site for the Src homology 2 (SH2) domain of Src or Fyn. Then Src phosphorylates FAK at Tyr^{925}, creating a binding site for the signaling complex which includes the adaptor Grb2 and Ras GTP-exchange factor mSOS. Grb2 can also indirectly link FAK phosphorylation at Tyr^{925} to ERK activation, via formation of a complex with Shc. These are interactions between signaling pathways that modify the organization of the cytoskeleton and ERK cascade. Thus, according to this mechanism, FAK would serve as an upstream regulator of MAP kinase activity. However, integrins can activate ERK independent of FAK activation. Being different from slow and sustained FAK mediated ERK activation, Shc might be responsible for the initial high level activation of ERK through a complex formation with Shc/Fyn/Cav-1 (228). Certain alpha integrins bind to the membrane protein caveolin-1 through their external and trans-membrane domains (89). The FAK independent activation of ERK by integrins appears to involve PI3K and PKC activation (228).

3.8.2. Integrin-mediated p38 and JNK activation

Although integrins couple to JNK and p38 activation (120, 227), the proximal signaling mechanisms remain to be fully explored. There is evidence that p38 and

JNK are activated by FAK dependent (10) and FAK independent mechanisms (120) in stretched cardiac myocytes (Figure 5). Integrins may also activate MAP kinases via cross-talk with other receptor systems. For example, it has recently been shown that $beta_1$ integrin plays a crucial role in beta-adrenergic receptor-stimulated myocardial remodeling with effects on cardiac myocyte hypertrophy, apoptosis and left ventricular function (229).

4. INTEGRIN CROSS-TALK WITH OTHER RECEPTOR SYSTEMS

Most types of cells require integrin-mediated attachment to extracellular matrix in order to respond to growth factor stimulation for proliferation and survival. Thus, the concept that integrins closely collaborate with growth factors in signal transduction has gradually emerged. The relatively close proximity of integrins and growth factors allows the formation of signaling complexes upon cell adhesion or growth factor stimulation. For example, activation and clustering promotes activation of growth factor receptors. Integrin-mediated clustering activates epidermal growth factor receptor (EGFR) in the absence EGF (230). Because integrins and growth factor receptors share many common elements in their signaling pathways, many opportunities exist for integrins and growth factors to engage in cross-talk. The most intensely studied pathways coordinately regulated by integrins and growth factor receptor tyrosine kinases (RTKs) have been at the level of focal adhesions, Rho GTPases, MAP kinases and transcriptional regulation.

Focal adhesions represent an important point of convergence for integrin and growth factor signaling and include key components activated by both systems, such as PKC, Src, FAK and ILK. Through the kinase domain of ILK, the cytoplasmic tails of $beta_1$ and $beta_3$ integrins are linked to the RTK, the actin cytoskeleton and other clustered integrins at the focal adhesion complex. Integrin signaling is coupled to growth factor signaling through the N-terminal ankyrin repeats of ILK bound to the LIM domains of PINCH, which bind to RTKs through the adaptor protein NCK2 (231). Thus, the PINCH-ILK-parvin complex serves to integrate signals from growth factors to the actin cytoskeleton, the ECM and to downstream signaling targets. The effects of integrin crosstalk with tyrosine kinases such as ILK, FAK and Src transduce the signal to small GTPases Ras, RhoA, Rac1 and Cdc42. The small GTPases function to further project the signal down a pathway that will either regulate the actin cytoskeleton or regulate cell proliferation. Ras is activated by adaptor proteins Shc and Grb2 after RTK phosphorylation by growth factors, and leads to the MAP kinase signaling pathway (232). RhoA may be activated from FAK, transducing the signal downstream directly to actin, which influences cell motility, stress fiber formation and filopodia/lamellipodia formation. Also, from FAK signaling through IP_3, the small GTPase Rac1 may be activated, which leads directly to the MAP kinase signaling. Cdc42 acts as an intermediate that links Rac1 to actin, and may be activated indirectly by stress such as cytotoxic drugs, irradiation, heat shock, reactive oxygen species, or lipopolysaccharide.

In general, MAP kinase signaling cascades are initiated after the small GTPases, and consist of convergence points at ERK, JNK, and p38 that direct the signaling pathways to certain transcription factors, and also cross-communicate through adaptor proteins. The MAP kinase signaling cascade that converges at ERK, is activated through the classical MAP kinase pathway by the binding of growth factors at RTKs, and proceeds through Ras. Addition of soluble mitogens to cells in suspension triggers weak or transient activation of the MAP kinases ERK1/2, as compared to strong and sustained ERK activity in adherent cells (233, 234). In this circumstance attachment of cells to ECM is critical to ERK activation, suggesting that coordinate regulation by integrins not only enhances, but may be required for growth factor signaling. One example of the downstream signaling effects of ERK is the activation of the transcription factor CREB, whose target gene expression is cyclin D1 and Myc, resulting in cell proliferation and differentiation. The JNK and p38 cascades, however, are generally associated by stress-activated stimuli. Specifically, the JNK signaling pathway may be stimulated through stress signals at Cdc42 or through cytokine receptors such as TNF-alpha and interleukin-1 (IL-1). Activation of the downstream transcription factor AP-1 results in tissue morphogenesis and targeted gene expression of MMP9, which will cause tissue invasion. The p38 cascade is activated by cytokines such as IL-1, FAS ligand and transforming growth factor-beta (TGF-beta). Examples of downstream apoptotic transcription factors include MAX and MEF2C, although p38 activation may also lead to activation of CREB (232).

Aside from MAP kinase signaling, transcription factors may also be triggered by the Akt signaling pathway. Akt promotes cell survival by inhibiting the pro-apoptotic proteins BAD, caspase-3 and caspase-9. Cell growth and angiogenesis are stimulated by activation of the downstream transcription factors NF-kappaB and HIF1, which allow targeted gene expression of VEGF, inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2), a pro-inflammatory mediator. The expression of these factors facilitates extracellular communication and provides an adaptive cell response based on information coordinated by integrins at the cell membrane (231). The cell response may also influence the expression of specific integrins to further modify specific aspects of chemical or mechanical signaling. For example, Ang II upregulates the expression of $beta_1$ integrins through activation of AT_1, resulting in the proliferation of cardiac fibroblasts that occurs during cardiac remodeling (7). In endothelial cells, signaling induced by VEGFR-2 is critical for $alpha_v$ $beta_3$ integrin-dependent cellular adhesion involved in angiogenesis (235).

Thus, in general, integrins synergize with growth factor pathways to enhance their activity. Detailed analysis of the FAK, MAP kinase and Rho GTPase pathways have revealed multiple points of intersection, suggesting a complex network of interactions (Figure 4 and 5). The importance of cytoskeletal organization and mechanical

tension for integrin signalling thereby provides a mechanism by which these considerations can influence the responses of cells to growth factors as well, providing a convergence point for different classes of extracellular cues. However, because many of the interactions between integrins and growth factor receptors are unique to specific cell types and integrins expressed, future studies are required to elucidate the specific mechanisms by which this mechanism is operational in the cardiovascular system.

5. THERAPEUTIC TARGETING OF INTEGRIN SIGNALING

Several integrin effectors been proposed to be potential therapeutic targets. However, targeting of a single molecule has limited therapeutic value due to the intensive cross-talk and redundancy of signals in the transduction network (230, 236, 237). A more novel and rational therapeutic approach may be to use a combination of multiple signaling inhibitors/activators, according to the molecular context of the disease process. However, this approach is limited by a lack of understanding as to how integrin receptors mediate cross-talk among the various signaling pathways. The unraveling of integrin signaling mechanisms will likely reveal new and more specific therapeutic targets for the treatment of cardiovascular disease.

5.1. ILK as a Therapeutic Target

As indicated above, recent advances in cardiac physiology have identified ILK as an essential molecule regulating cardiac growth, contractility, and repair (160, 163). As a result, attempts are being made to develop therapeutic agents which can effectively target this molecule (238). Pharmacologic modulation of the ILK or the PINCH-ILK-parvin complex may be useful for treating cardiovascular diseases such as cardiac hypertrophy and atherosclerosis (239). Drugs targeted to integrin or ILK may block virus infection and attenuate the symptoms of viral myocarditis (240). In contrast, ILK activation can promote cardiac repair after myocardial infarction, and thus development of agents that stimulates ILK activity or expression would provide a novel approach for therapeutic interventions post–myocardial infarction. Given the central role of ILK in cardiovascular pathophysiology, development of therapeutic agents which can be used to modulate ILK may prove to be a worthwhile area of endeavor.

5.2. MAP kinases as therapeutic targets

5.2.1. p38 inhibitors

In the past two decades, several p38 inhibitors (241-245) have been used to understand the role of this kinase in a variety of tissues. Small-molecule inhibitors of p38 reduces vascular injury in the rabbit (246) and block cardiac hypertrophy and remodeling in hypertensive stroke-prone rats (247), in rats and mice subjected to myocardial infarction (248, 249), cardiomyopathic hamsters (250) and in mice expressing dominant-negative 14-3-3 chaperone protein in the heart (251). In humans, these agents are potent inhibitors of TNF-alpha and other pro-inflammatory cytokines. A number of clinical trials are currently underway with these agents for non-cardiovascular indications such as rheumatoid arthritis and Crohn's disease, as well as cardiovascular indications such as atherosclerosis. Some of these compounds have reached phase II and III trials (252). However, despite the enthusiasm of various pharmaceutical companies to develop MAP kinase inhibitors, in several cases clinical trials have been stopped due to safety concerns. One of the underlying reasons for these undesirable effects might be the cross-reactivities against other kinases or other cellular signaling molecules. Almost all p38 alpha/beta inhibitors are ATP competitors, which have a nonspecific effect of targeting other kinases. Thus, the development of inhibitors which target p38 in a more unique manner may improve the selectivity profile of these agents. The finding that p38 can be activated by its association with TAB1alpha (253) suggests that inhibitors designed to disrupt this interaction may have distinct advantages over ATP competitors. Thus, an alternative approach might be to target other molecules in the p38 pathway. Another complication is that MAP kinases have broad expression profiles and engage in complex cross-talk and feedback loops (254, 255) that finely control cellular functions. This may raise unexpected complications for single-kinase inhibition and may be another explanation for why many p38 inhibitors have failed in clinical trials.

5.2.2. JNK inhibitors

There has been enthusiasm for pharmaceutical companies to develop JNK inhibitors since these signaling factors are involved in a variety of diseases including diabetes, atherosclerosis, stroke, and Parkinson's and Alzheimer's disease. However, the development of JNK inhibitors for clinical therapies has been met with several obstacles. The lack of specificity is an important concern since current JNK inhbitors used in clinical trials block JNK1, JNK2 and JNK3. Although inhibition of JNK may suppress many of the pathological features of these diseases, prolonged use of these agents would be expected to adversely affect normal physiological function in other organs. One of the organs which could be adversely affected by JNK inhibitor therapy is the heart. Although a number of *in-vitro* studies have suggested that JNK is prohypertrophic in cardiac myocytes (219, 256-258), this postulate has been recently challanged by results from several *in vivo* studies (219, 250, 258-261) which indicate that JNK is cardioprotective. In mice, selective deletions of JNK1, JNK2 and JNK3 have demonstrated that JNK1 is required to preserve cardiac function in the early response to pressure overload (262). In addition, JNK signaling has been shown to have a protective role in cardiac patho-physiology during a variety of stresses like oxidative stress (263) and reperfusion (264). Thus, JNKs are interesting and promising targets. However, because of their relevance to cell biology in general, it is not sufficient to block specifically either all JNKs or individual isoforms, thus limiting the use of JNK as a drug target. It is important to emphasize that small molecular JNK inhibitors are now being used in clinical trials for the treatment of autoimmune, inflammatory, and neurodegenerative diseases (265). There are potential risks related to such novel therapies, in particular when hypertensive and/or

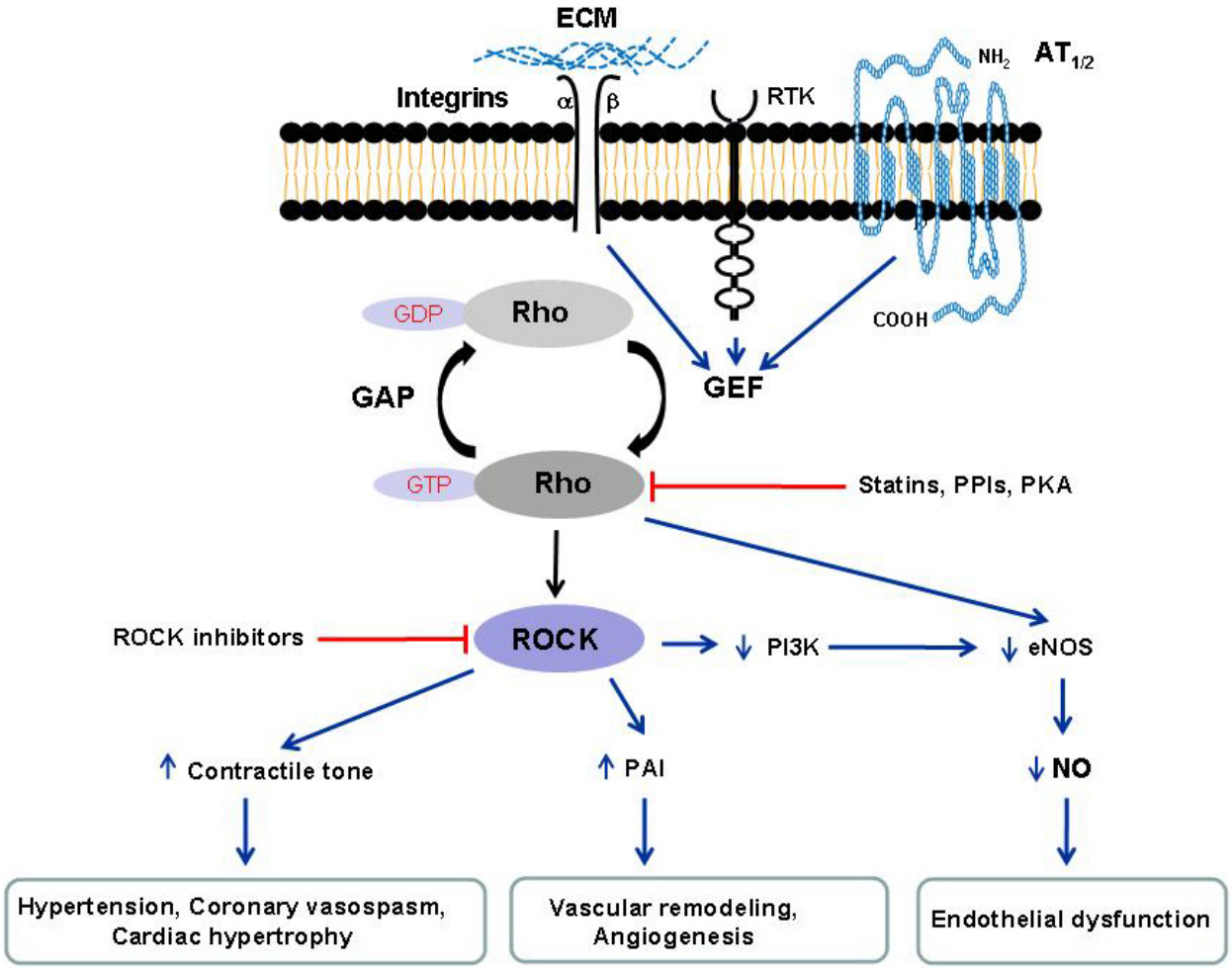

Figure 6. Integrin Mediated MAP Kinase Signaling. Mechanical stretch and shear stress are important regulators of function in the cardiovascular system. Mechanical forces are detected by mechanosensors (integrins, RTKs, stretch-activated channels) which activate signal transduction cascades involving Rho GTPases (RhoA, Rac1, Cdc42), MAP kinases (ERK1/2, JNK, p38) and subsequent transcription factors which regulate the function of cardiac myocytes, fibroblasts, platelets, and vascular smooth muscle cells. Integrin receptor engagement by extra-cellular matrix (ECM) such as collagen or fibronectin or vitronectin stimulates FAK at Tyr^{397}, Tyr^{925} and Tyr^{806}. Activation of FAK at Tyr^{397} creates a binding site for the SH2 domain of c-Src which can further phosphorylate FAK at Tyr^{925}. Activation of FAK and Src-family PTKs can activates Shc tyrosine phospholylation at Tyr^{317} which leads to the activation of Grb2. Activation of Grb2 can potentiate the translocation of the GDP-GTP exchange protein Sos to the plasma membrane, leading to enhance GTP exchange on RAS. Activation of the Erk cascade is one target for the activated Ras through Raf and MEK1/2. Association of Src-family kinases with FAK also potentiates the tyrosine phosphorylation of p130Cas, which leads to activation of the JNK MAP kinase cascade. The p38 pathway can be activated by several integrin dependent upstream signaling molecules or crosstalk such as FAK/PI3K/Rac1/p38 or FAK/RhoA/p38, which lead to cardiac hypertrophy and apoptosis. Cav-1: caveolin-1; Sos: Son of sevenless guanine nucleotide exchange factor; Pak: p21-activated kinase; MEK: MAPK/Erk kinase; TAK: TGF beta-activated kinase; ERK: Extracellular signal-regulated kinase; JNK: Jun N-terminal kinase ; SAPK: Stress-activated protein kinase; Src: Rous sarcoma oncogene cellular homolog; PI3K: phosphatidylinositol 3-kinase; Shc: SH2-containing collagen-related proteins; Nck-2: Nck adaptor protein ; Cas: Crk associated substrate; GRB2: Growth factor receptor-bound protein.

heart failure patients are included in the study, and it is recommended that serial evaluation of cardiac function should be monitored during trial/treatment (262).

5.3. Rho GTPases as therapeutic targets

Work in the past decade has clearly demonstrated that Rho GTPases have key roles in the development of atherosclerosis and cardiac hypertrophy. Thus, the importance of targeting Rho GTPases in the cardiovascular system is now recognized as a therapeutic strategy. To date, the major classes of agents which target Rho GTPase signaling include Rho kinase (ROCK) inhibitors, hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase

inhibitors (statins) and protein prenyltransferase inhibitors (Figure 6).

5.3.1. Rho-kinase (ROCK) inhibitors

Fasudil hydrochloride (1- (5-isoquinolinesulfonyl) homopiperazine dihydrochloride or HA-1077), originally developed as a calcium channel inhibitor, inhibits Rho-kinase by competing with ATP for the binding site of the kinase catalytic subunit (266). Intracoronary administration of fasudil attenuates both arterial spasm and the extent of subsequent myocardial ischemia in patients with vasospastic angina (267). Fasudil is converted *in vivo* to its active metabolite hydroxyfasudil; a more-selective inhibitor of ROCK, which might represent an even better drug for clinical development. However, the future of fasudil and hydroxyfasudil in the clinic is uncertain since both agents exert nonspecific inhibitor effects on other Ser/Thr kinases, such as PKA and PKC. An effort has been made to develop more-specific and more potent ROCK inhibitors. This has resulted in development of (hexahydro-1- (isoquinoline-5-sulfonyl)-1H-1, 4-diazepine) and H1152A, which are refinements of the fasudil structure.

Another ROCK inhibitor is Y-27632 ((+)- (R)-trans-N- (4-pyridyl)-4- (1-aminoethyl)-cyclohexanecarboxamide), which is more specific and potent than fasudil. Like fasudil, Y-27632 inhibits ROCK by competing with ATP for binding to the catalytic site of the kinase (268). The demonstration that Y-27632 can lower blood pressure in three models of hypertension provided the first link between ROCK and development of cardiovascular disease (202). More recently, Y-27632 has also been used as an important pharmacological tool for elucidating the importance of ROCK in pulmonary hypertension (269) and other disease processes such as tumor cell invasion and asthma (270, 271). In the post-infarcted myocardium, Y-27632 decreases both basal and 5-hydroxytryptamine induced contractile responses (272), suggesting that ROCK contribute towards maintaining a contractile function in the failing heart. Although Y-27632 has a reasonably selective profile, it also inhibits Rho dependent PKC-related kinase 2 (PRK-2), with potency similar to ROCK2. Thus, its use as a therapeutic agent will remains uncertain until its safety profile has been carefully studied. Despite these potential limitations, it remains as an important pharmacological tool which can be used to examine the involvement of ROCK in the pathogenesis of cardiovascular and other disorders. Recently, SLx2119, a highly selective and potent ROCK2 inhibitor has been developed. Administration of SLx-2119 attenuates arterial plaque formation in apolipoprotein-E deficient mice, suggesting that selective ROCK inhibition could be used to limit atherosclerosis and avoid unwanted hemodynamic side-effects, as compared to other ROCK inhibitors (273).

5.3.2. Statins

Statins represent the first HMG-CoA reductase inhibitors, which were identified in 1976 (274). Statins are powerful hypolipidemic drugs widely used to lower elevated plasma cholesterol levels. Although statins exert cardio-protection primarily through its hypolipidemic nature, results from clinical trials have revealed that statins have beneficial effects on the cardiovascular system, even in normocholesterolemic individuals. The cholesterol-independent cardiovascular benefits of statin therapy have been attributed to their effects on endothelium, which can occur within 24 h of treatment (275). In blocking cholesterol biosynthesis, statins also prevent the formation of isoprenoid intermediates, including geranylgeranyl pyrophosphate, which is required for the geranylgeranylation of RhoA. The isoprenylation is a prerequisite for its activation, facilitating its interaction with the plasma membrane where GDP-GTP exchange occurs. By preventing this membrane interaction, statins inactivates RhoA, leading to increased eNOS expression and activity, and increased endothelial NO production (276). Recent functional evidence consistent with this is that both *in vivo* and *in vitro* chronic statin treatment reduces the vascular contractility of isolated intact mesenteric artery via increased NO production (277). Although the impact of statin therapy in cardiovascular disease appears to be predominantly vascular, recent animal (278-282) and human studies (283) suggest that statins may also have direct beneficial effects on the myocardium. Inhibition of small GTP-binding protein RhoA might be one of the cholesterol-independent mechanisms of statin mediated cardio-protection. However, because statins have broad specificity and multiple cellular targets, the safety of these drugs in heart failure patients remains to be addressed prior to routine use in these individuals. Several large-scale prospective outcome studies in heart failure are underway (283), which are likely to provide difinitive answers regarding the utility of these drugs for the treatment of heart disease.

6. SUMMARY AND PERSPECTIVES

In summary, it is clear that integrins play key signaling roles in the cardiovascular system. A substantial amount of work is required to clarify the mechanisms by which integrins function in cardiac and vascular cells. It remains to be determined how various integrin receptors couple to proximal effectors and cross-talk with other mechanosensing and growth factor receptors in all the cardiovascular cell types. Novel theoretical and experimental methodologies will be required to unravel the precise details of these signaling mechanisms. A better understanding of integrin signaling should aid in the development of new therapeutic strategies and approaches for the treatment of cardiovascular disease.

7. ACKNOWLEDGMENT

This work was supported by grants from National Institutes of Health (HL-68838) and Scott and White Memorial Hospital.

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Abbreviations: AIAC: Actin-integrin adhesion complex; AKT: Protein kinase B; CAS: p130 Crk associated substrate; Cav-1: Caveolin-1; CFBs: Cardiac fibroblasts; cRGD: Cyclic Arginine-Glycine-Aspartic acid peptide; ECs: Endothelial cells; ECM: Extracellular matrix; eNOS: Endothelial nitric oxide synthase; ERK: Extracellular signal-regulated kinase; FA: Focal adhesion; FAK: Focal adhesion kinase; FAT domain: Focal adhesion targeting domain; FBs: Fibrillar adhesions; FN: Fibronectin; FRNK:

FAK related non-kinase; FXs: Focal complexes; GAPs: GTPase activating proteins; GDIs: GDP dissociation inhibitors; GPI: Glycosylphosphatidylinositol; GPCRs: G-protein coupled receptors; GSK3beta: Glycogen synthase kinase–3□; I-domain: Inserted domain; ILK: Integrin-linked kinase; ILKAP: Integrin-linked kinase-associated phosphatase 2C; IQGAP: Calmodulin-binding GTPase activating proteins; JNK: c-jun N-terminal kinase; LV: Left ventricle; MAP Kinase: Mitogen-activated protein kinase; MMP: Matrix metalloproteinase; PAK: p21-Activated kinase; PH: Pleckstrin homology; PI3K: Phosphoinositide 3-kinase; PINCH: Particularly interesting new cysteine-histidine rich protein; PIP3: Phosphatidylinositol 3,4,5-triphosphate; PKA: Protein kinase A; PTEN: phosphatase and tensin homolouge deleted on chromosome ten; RGD: Arginine-Glycine-Aspartic acid; ROCK: Rho kinase; SAPKs: Stress-activated protein kinases; VSM: Vascular smooth muscle; WASP: Wiskott-Aldrich syndrome protein; WAVE: WASP with a V-domain.

Key Words: Cardiac hypertrophy Cardiomyocytes, Fibroblasts, Caveolin, Integrins, Focal-adhesion kinase, Integrin-linked kinase, Mitogen-Activated Protein Kinase, Review

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